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Domestic Animal Endocrinology 40 (2011) 129–138
DOMESTIC ANIMAL ENDOCRINOLOGY
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# Acute handling disturbance modulates plasma insulin-like growth factor binding proteins in rainbow trout (*Oncorhynchus mykiss*)

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Received 21 June 2010; received in revised form 22 September 2010; accepted 24 September 2010

**Abstract**

The effects of acute stressor exposure on proximal (growth hormone [GH]) and distal (insulin-like growth factor-I [IFG-I] and insulin-like growth factor-binding proteins [IFGBPs]) components of the somatotropic axis are poorly understood in finfish. Rainbow trout (*Oncorhynchus mykiss*) were exposed to a 5-min handling disturbance to mimic an acute stressor episode, and levels of plasma GH, IGF-I, and IGFBPs at 0, 1, 4, and 24 h post-stressor exposure were measured. An unstressed group was also sampled at the same clock times (09:00, 10:00, 13:00, and 08:00 [the following day]) as acute stress sampling to determine temporal changes in the above somatotropic axis components. The acute stressor transiently elevated plasma cortisol and glucose levels at 1 and 4 h post-stressor exposure, whereas no changes were seen in the unstressed group. Plasma GH levels were not affected by handling stress or sampling time in the unstressed animals. Plasma IGF-I levels were significantly depressed at 1 and 4 h post-stressor exposure, but no discernible temporal pattern was seen in the unstressed animals. Using a western ligand blotting technique, we detected plasma IGFBPs of 21, 32, 42, and 50 kDa in size. The plasma levels of the lower-molecular-weight IGFBPs (21 and 32 kDa) were unaffected by handling stressor, nor were there any discernible temporal patterns in the unstressed animals. By contrast, the higher-molecular-weight IGFBPs (42 and 50 kDa) were affected by stress or time of sampling. Levels of the 42-kDa IGFBP levels significantly decreased over the sampling period in unstressed control animals, but this temporal drop was eliminated in stressed animals. Levels of the 50-kDa IGFBPs also decreased significantly over the sampling time in unstressed trout, whereas handling disturbance transiently increased levels of this IGFBP at 1 h but not at 4 and 24 h post-stressor exposure compared with the control group. Overall, our results suggest that acute stress adaptation involves modulation of plasma IGF-1 and high-molecular-mass IGFBP levels (42 and 50 kDa) in rainbow trout.


*Keywords:* Growth hormone; Insulin-like growth factor-I; Insulin-like growth factor binding protein; Stress; Rainbow trout

## 1. Introduction

Growth in finfish is controlled by the endocrine growth axis (somatotropic axis), which involves the secretion of pituitary growth hormone (GH) and the hepatic production of its anabolic intermediary, insulin-like growth factor-I (IGF-I) [1–3]. The importance of the GH-IGF axis to teleost growth has been repeatedly demonstrated in a number of finfish species [4]. More importantly, in studies where both hormones (GH and IGF-I) have been measured in growing fish, positive correlations between GH and IGF-I and IGF-I and growth have been reported [5–9]. These correlative

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doi:10.1016/j.domaniend.2010.09.007

findings suggest that IGF-I levels may be a suitable indicator of growth status in captive/culture finfish populations [10,11] and recent studies have further suggested that IGF-I levels may also be used as a surrogate measurement to ascertain the effective level of nutrition (eg, nutritional plane) and the efficacy of new diet formulations [5,11–13].

Despite the positive association with IGF-I and growth, the biological actions of IGF-I are further modified by high-affinity binding proteins called insulin-like growth factor binding-proteins (IGFBPs) that are also found in the blood. The IGFBPs (more than 6 have been identified in mammals) bind IGF-I with high affinity and influence (typically increasing) the half-life of IGF-I in the blood, the distribution of IGF-I within and between tissue compartments, and the interactions of IGF-I with its receptor(s) on target tissues [14,15]. Four putative mammalian homologues have been identified in the plasma and serum of teleosts using immunoassays, ligand blotting, and chromatography methods [3,16–20], whereas additional IGFBPs have been identified and characterized via genomic techniques [21–26]. Physiologically, low-molecular-weight IGFBPs (<32 kDa) are elevated in catabolic states such as fasting, hypoxia, or salinity challenge and are thus associated with impaired metabolism and growth [18,27–34]. By contrast, the higher-molecular-weight IGFBPs (>32 kDa) are elevated in anabolic states such as positive nutritional plane or other situations where elevated GH levels are seen and are thus associated with high growth [5,18,19,31,34]. Given the emerging relationships between IGF-I and the IGFBPs, as well as the potential for IGFBPs to exert IGF-independent actions [14,15], there is continued interest in understanding the function and regulation of these important proteins in teleosts.

The somatotropic axis can be modulated and growth affected by environmental and nutritional factors [3,4,35]. More importantly, exposure to stressors seen in captive rearing conditions (poor nutrition and handling and confinement) can negatively impact growth and well-being via alteration of GH and IGF-I (and cortisol) levels [36–40]. The effect of such long-term stressors on the somatotropic axis has been characterized in a wide range of commercially important teleosts including channel catfish [26,41], rabbit fish [42], rainbow trout [9,10,43], salmon [6,37], sea bream [12,44], and tilapia [7], to name a few. By contrast, there are only a few studies that have examined how short-term (<10 min) handling and confinement stress influence GH or IGF-I and the IGFBPs in finfish [45,46], and species differences make generalizations difficult. More importantly, studies that have been designed to evaluate the response of the somatotropic axis prior to and during the recovery from an acute stressor, wherein cortisol and glucose homeostasis were established, are lacking. Against this background, we have evaluated the effects of a 5-min handling disturbance, mimicking routine handling (husbandry) practices, on plasma GH, IGF-I, and IGFBPs levels in rainbow trout.

## 2. Materials and methods

### 2.1. Animals

Juvenile rainbow trout were obtained (~227 g body mass) from Humber Springs trout hatchery (Mono Mills, Ontario, Canada) and acclimated in 2,000-L tanks with continuous flow-through water at 13 °C and 12L:12D photoperiod for 3 wk prior to experimental treatment. Animals were fed to satiation (3.0 mm sinking feed; Martin Mills, Inc, Elmira, Ontario, Canada) once daily 5 d a week. All work was done in accordance with approved animal care protocols with the University of Waterloo.

### 2.2. Experimental protocol

Animals were subjected to a standardized stress protocol as previously described [47]. For the acute stress study, 24 trout were randomly distributed into three 100-L tanks (8 fish per tank) and further acclimated for 2 wk. Tanks were randomly assigned to 1 of 3 sampling time points of 1, 4, and 24 h after stressor exposure. Animals were fed to satiation once daily during this acclimation period. Food was withheld from animals for 24 h prior to experimental manipulation and throughout the sampling period. Following acclimation and the 24-h food withdrawal, 2 animals from each tank (6 fish total) were initially sampled to provide a 0 time (pre-stress) control at 09:00. The remaining animals (6 animals per tank, 18 animals total) were immediately subjected to a standardized stressor of netting and chasing for 5 min and then left undisturbed until they were sampled at 1 h (10:00), 4 h (13:00), and 24 h (08:00 the following day) after stressor exposure. At the time of sampling, the remaining animals were quickly netted from each separate tank (1, 4, or 24 h post-stress) and anesthetized and blood was collected as described below. To ensure that sampling time did not affect the measured parameters in the stressed animals, a second group of fish (5 animals per tank, 20 total animals) was randomly distributed into 4 identical 100-L tanks to

serve as unstressed controls and allowed to acclimate for 1 wk. For this experiment, tanks were assigned to sampling times (clock time; see below) corresponding to the same times at which the stressed animals were sampled (0, 1, 4, and 24 h). Following the acclimation period, food was withheld for 24 h and the unstressed animals were sampled. Sampling consisted of quickly netting all animals from each (separate) tank at clock times of 09:00 (0 h), 10:00 (1 h), 13:00 (4 h), and 08:00 (the following day; 24 h). For blood sampling in both studies, animals were quickly anesthetized with 2-phenoxyethanol (1:1,000) and blood was collected (all animals within 3 min) by caudal puncture into heparinzed tubes. Plasma was collected by centrifugation (6000*g* for 10 min), aliquoted, and stored at −80 °C until analyses. Plasma cortisol and glucose levels for this study have been previously reported [47].

### 2.3. Plasma GH and IGF-I levels

Plasma GH levels were determined using $^{125}$I-labeled recombinant salmon (rs) GH (Lot No. DAB-GHB1; GroPep, S. Australia, Australia) for label, unlabeled rsGH as standards, and antisalmon GH primary antibody (Lot No. AJI-PAN1; GroPep) as established in our laboratory [31,34]. Inter- and intra-assay coefficients of variation were 7.0% and 7.6%, respectively. The detection limit of the assay, defined as 90% binding, was 0.45 ± 0.14 ng/mL. Twenty-five microliters of whole plasma was used for each assay and samples were measured in duplicate.

Plasma IGF-I levels were determined according to the method of Shimizu et al. [20] as established in our laboratory [31,34]. We used $^{125}$I-labeled rsIGF-I (Lot No. AJ1-PAN1; GroPep Pty, Ltd, Adelaide, South Australia, Australia) for label, unlabeled rIGF-I for standards, and antibarramundi IGF-I primary antibody (Lot No. DAA-PAF1) (GroPep, Ltd) for this assay. Inter- and Intra-assay coefficients of variation were 5% and 4%, respectively. The detection limit of the assay, defined as 90% binding, was 0.1 ± 0.01 ng/mL. Samples consisted of acid-ethanol–extracted plasma and were measured in triplicate.

### 2.4. Plasma IGFBPs

Western ligand blotting using DIG labeled-rsIGF-I was done according to the method of Shimizu et al. [20] as modified, validated, and described by Shepherd et al. [31,34] for measurement of IGFBPs in rainbow trout plasma. Plasma samples were measured in duplicate. In addition, a plasma pool was run on each gel/blot and values for IGFBP bands in experimental plasma samples were normalized to related values in the plasma pool to control for blot-to-blot variation in band intensities. Values are represented as arbitrary densitometry units (ADU).

### 2.5. Statistical analysis

Differences among groups within each experiment (stressed or unstressed) were evaluated separately by 1-way analysis of variance (ANOVA; SigmaPlot version 11.0, San Jose, CA, USA) with sampling time(s) as the independent variable. Given that the 2 experiments (stress and unstressed) were conducted separately and with different numbers of tanks and animals per group, each experiment has been separately analyzed via 1-way ANOVA. As such, this precludes pairwise comparisons of measured parameters (between experiments) that could be made following a 2-way ANOVA statistical model. Additionally, given that our experimental approach treated the individual animals as the experimental unit and did not incorporate replicate tanks, we are unable to determine whether there was a tank effect on the parameters measured. Where significant differences occurred ($P < 0.05$) following 1-way ANOVA, comparisons between groups (time points within each experiment) were performed using Fisher's protected least significant difference (FPLSD) test for pairwise comparisons [48]. The SigmaPlot statistical package was used for statistical analyses. Stated $P$ values for individual comparisons are general cut-off ($P < 0.05$) values using a 2-tail alternative.

## 3. Results

### 3.1. Plasma cortisol and glucose

Plasma cortisol and glucose levels were significantly elevated at 1 and 4 h post-stressor exposure and returned to pre-stressor levels by 24 h. Neither plasma cortisol nor glucose levels in unstressed animals were significantly different over the sampling time (see [47] for results).

### 3.2. Plasma GH and IGF-I

There was no significant ($P = 0.922$) effect of handling disturbance on plasma GH levels in stressed animals (Fig. 1A), nor were there any significant ($P = 0.579$) changes in plasma GH levels associated with sampling time in unstressed animals (Fig. 1B).

Handling disturbance significantly ($P = 0.038$) affected plasma IGF-I levels over the sampling time course. Pairwise comparisons show that plasma IGF-I

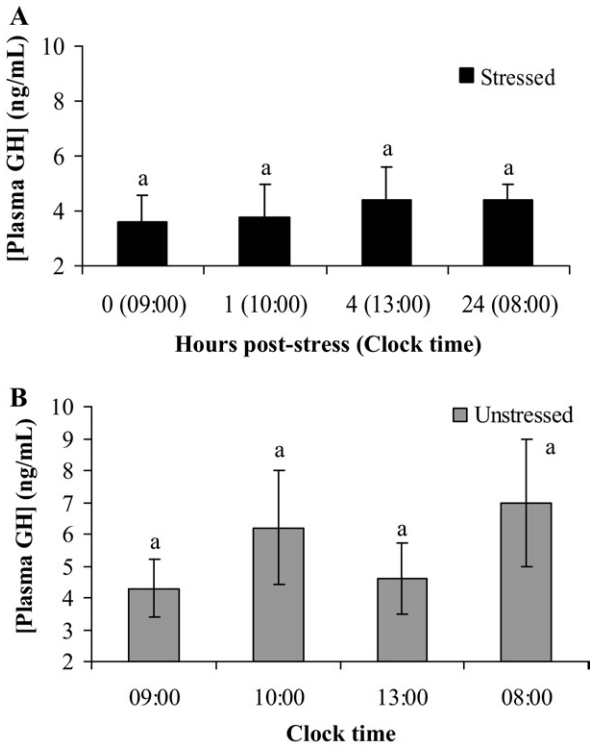


Fig. 1. Time course of plasma growth hormone (GH) levels in rainbow trout subjected to a handling disturbance (A) and in nonstressed (B) animals. Stressed animals were subjected to a 5-min handling stress (netting and chasing), whereas unstressed animals were not handled. Stressed animals were sampled at 0 (before stressor/handling), 1, 4, and 24 h after handling disturbance. Values in parenthesis (x-axis labels) represent the clock time at which the sampling time points occurred. To control for temporal variation, unstressed animals were sampled at 09:00, 10:00, 13:00, and 08:00 (the following day; clock time) and these times corresponded with sampling times in the handled (stressed) animals. Units are nanograms per milliliter ± SEM (n = 5–6 fish). There were no significant differences between sampling times on plasma GH levels in stressed ($P = 0.922$; 1-way ANOVA) or unstressed ($P = 0.579$; 1-way ANOVA) animals. Time points (groups) with different letters are significantly different ($P < 0.05$; FPLSD).

levels were significantly ($P < 0.05$) depressed at 1 and 4 h post-stressor exposure (compared with 0-time controls), but levels had returned to control (time 0) values by 24 h (Fig. 2A). In animals from the unstressed study, plasma IGF-I levels did not significantly ($P = 0.710$) differ over the sampling time course (Fig. 2B).

### 3.3. Plasma IGFBPs

Four distinct IGFBPs (21, 32, 42, and 50 kDa) were detected in the plasma of rainbow trout from this study as in previous studies [31,34]. There were no significant effects of handling disturbance (Figs. 3A and 4A) on plasma levels of either the 21-kDa ($P = 0.53$) or the 32-kDa ($P = 0.71$) IGFBPs over the sampling time course. Similarly, were there no significant effects of sampling time on plasma levels of the 21-kDa ($P = 0.23$) or 32-kDa ($P = 0.98$) IGFBPs in unstressed animals (Figs. 3B and 4B). Plasma levels of the 42-kDa IGFBP were not significantly ($P = 0.12$) affected by handling disturbance (Fig. 5A) over the sampling time course, although mean levels did increase over time. In unstressed animals, levels of the 42-kDa IGFBP were significantly ($P < 0.001$) affected by sampling time. Pairwise comparisons revealed that plasma levels of the

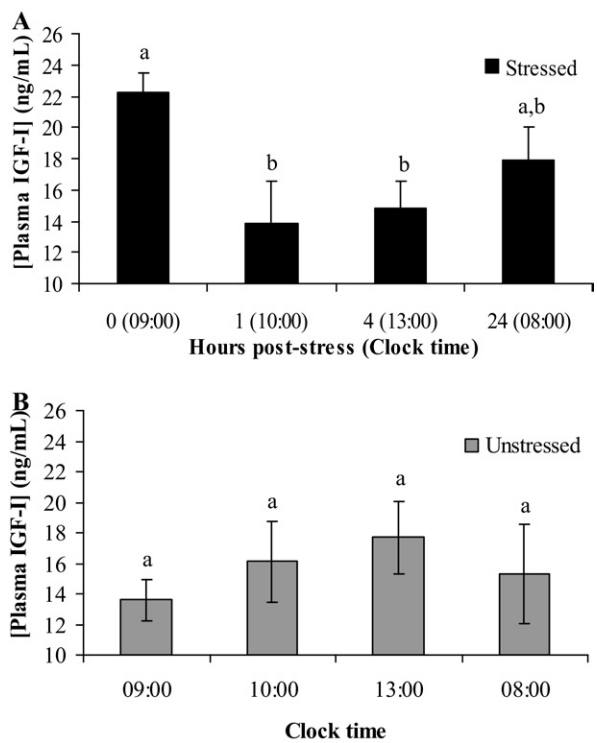


Fig. 2. Time course of plasma insulin-like growth factor-I (IGF-I) levels in rainbow trout subjected to a handling disturbance (A) and in nonstressed (B) animals. Stressed animals were subjected to a 5-min handling stress (netting and chasing), whereas unstressed animals were not handled. Stressed animals were sampled at 0 (before stressor/handling), 1, 4, and 24 h after handling disturbance. Values in parenthesis (x-axis labels) represent the clock time at which the sampling time points occurred. To control for temporal variation, unstressed animals were sampled at 09:00, 10:00, 13:00, and 08:00 (the following day; clock time) and these times corresponded with sampling times in the handled (stressed) animals. Units are nanograms per milliliter ± SEM (n = 5–6 fish). There was a significant ($P = 0.038$; 1-way ANOVA) effect of sampling time on post-stress IGF-I levels in the stressed group (A), whereas there was no effect ($P = 0.71$; 1-way ANOVA) of sampling time on plasma IGF-I levels in the unstressed animals (B). Time points (groups) with different letters are significantly different ($P < 0.05$; FPLSD).

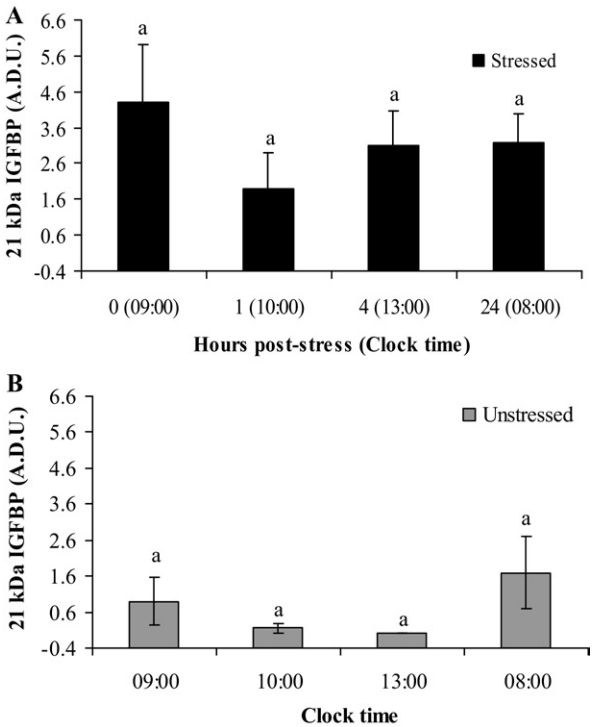


Fig. 3. Time course of the 21-kDa IGFBP in plasma of rainbow trout subjected to a handling disturbance (A) and nonstressed (B) animals. Stressed animals were sampled at 0 (before stressor/handling), 1, 4, and 24 h after handling disturbance. Values in parenthesis (x-axis labels) represent the clock time at which the sampling time points occurred. To control for temporal variation, unstressed animals were sampled at 09:00, 10:00, 13:00, and 08:00 (the following day; clock time) and these times corresponded with sampling times in the handled (stressed) animals. Units are arbitrary densitometry units (ADU) ± SEM. There were no significant effects of sampling time on levels of the 21-kDa IGFBP in stressed ($P = 0.53$; 1-way ANOVA) and unstressed ($P = 0.22$; 1-way ANOVA) animals. Time points (groups) with different letters are significantly different ($P < 0.05$; FPLSD).

42-kDa IGFBP were significantly ($P < 0.05$) lower at 10:00, 13:00, and 08:00 (the following day) compared with animals initially sampled at 09:00 (Fig. 5B). Plasma levels of the 50-kDa IGFBP were significantly ($P = 0.036$) affected by handling disturbance (Fig. 6A) over the sampling time course. In stressed animals, pairwise comparisons revealed that plasma levels of the 50-kDa IGFBP were significantly ($P < 0.05$) higher at 1 h after stressor exposure (compared with 0-time controls), but not at 4 h or 24 h poststress (Fig. 6A). In the unstressed animals, plasma levels of the 50-kDa IGFBP were significantly ($P < 0.001$) affected by sampling time (Fig. 6B). Pairwise comparisons revealed that plasma levels of the 50-kDa IGFBP in unstressed animals were significantly ($P < 0.05$) lower at 10:00, 13:00, and 08:00 (the following day) compared with animals initially sampled at 09:00 (Fig. 6B).

## 4. Discussion

Our results demonstrate for the first time that acute stress modulates plasma IGFBP levels in trout. Most studies on IGFBPs in fish have focused on their role in relation to feeding and fasting and very little is known about acute stressor(s) regulation of these proteins. We are only aware of a single study that examined the effects of acute handling stress on plasma IGFBPs in hybrid bass, but the stressor employed was of a longer duration and the IGFBPs in this species were of lower

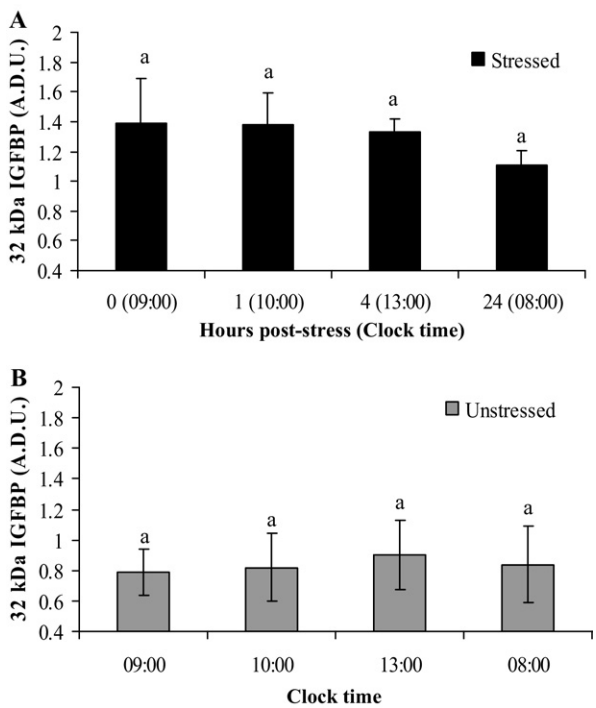


Fig. 4. Time course of the 32-kDa IGFBP in plasma of rainbow trout subjected to a handling disturbance (A) and nonstressed (B) animals. Stressed animals were subjected to a 5-min handling disturbance (netting and chasing), whereas unstressed animals were not handled. Stressed animals were sampled at 0 (before stressor/handling), 1, 4, and 24 h after handling disturbance. Values in parenthesis (x-axis labels) represent the clock time at which the sampling time points occurred. To control for temporal variation, unstressed animals were sampled at 09:00, 10:00, 13:00, and 08:00 (the following day; clock time) and these times corresponded with sampling times in the handled (stressed) animals. Units are arbitrary densitometry units (ADU) ± SEM. There were no significant effects of sampling time on levels of the 32-kDa IGFBP in stressed ($P = 0.71$; 1-way ANOVA) and unstressed ($P = 0.98$; 1-way ANOVA) animals. Time points (groups) with different letters are statistically different ($P < 0.05$; FPLSD).

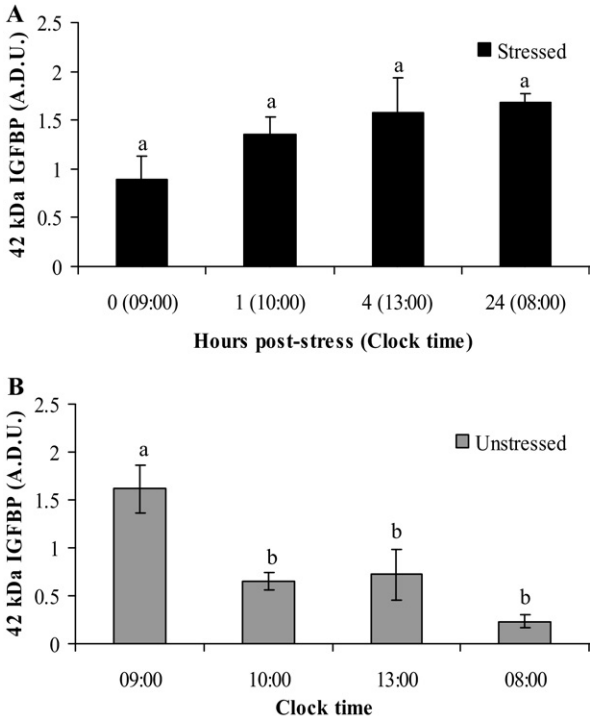


Fig. 5. Time course of the 42-kDa IGFBP in plasma of rainbow subjected to a handling disturbance (A) and nonstressed (B) animals. Stressed animals were subjected to a 5-min handling disturbance (netting and chasing), whereas unstressed animals were not handled. Stressed animals were sampled at 0 (before stressor/handling), 1, 4, and 24 h after handling disturbance. Values in parenthesis (x-axis labels) represent the clock time at which the sampling time points occurred. To control for temporal variation, unstressed animals were sampled at 09:00, 10:00, 13:00, and 08:00 (the following day; clock time) and these times corresponded with sampling times in the handled (stressed) animals. Units are arbitrary densitometry units (ADU) ± SEM. There were no significant effects of sampling time on levels of the 42-kDa IGFBP in stressed ($P = 0.121$; 1-way ANOVA), whereas there was a significant ($P < 0.001$; 1-way ANOVA) effect of sampling time in the unstressed control animals. Time points (groups) with different letters are statistically different ($P < 0.05$; FPLSD).

molecular weight (<35 kDa) [45], thus making direct comparisons difficult. We identified 4 IGFBPs of 21, 32, 42, and 50 kDa, which corresponds well with previous reports of IGFBPs in rainbow trout using a non-isotopic western ligand blotting method [31,34]. Although the lower-molecular-mass IGFBPs were not significantly affected by the handling disturbance, there were transient changes in the high-molecular-mass IGFBPs (>40 kDa), suggesting a role for these proteins in stressor-mediated IGF-I regulation in rainbow trout.

Given the link between GH and IGF-I in the growth of animals [4], a number of recent studies have examined how these hormones respond to acute stress in commercially important finfish species [9,12,37,44,45,49]. In the current study, we found no effect of a brief handling stressor on plasma GH levels, which is in agreement with an earlier study in rainbow trout that also employed a brief (<10 min) handling stressor [50]. By contrast, studies that employed longer-term stressors (greater than 10 min) have reported more variable results. Pickering and co-workers [39] found that a 1-h acute handling/crowding stressor and chronic (up to 24 h) crowding stressors reduced plasma GH in rainbow trout. By contrast, Kakizawa and co-workers [43] reported that plasma GH levels were unaffected by an 8-min exhaustive exercise (net chasing), but levels increased following a 30-min confinement stressor. More

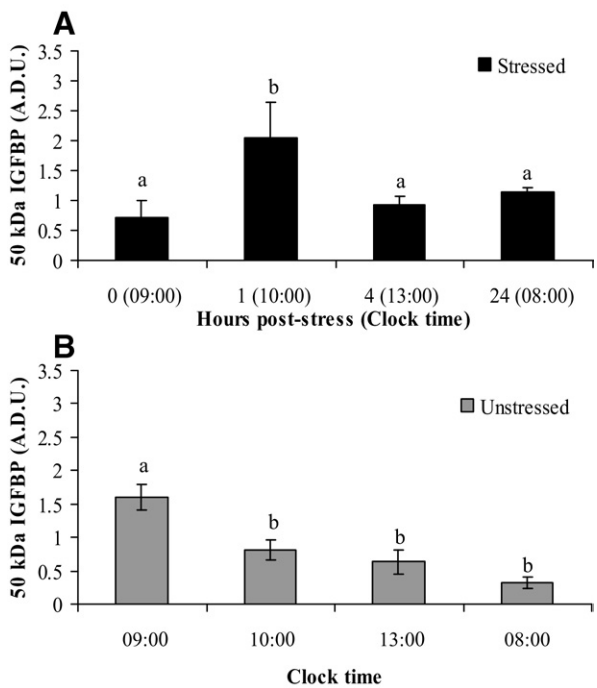


Fig. 6. Time course of the 50-kDa IGFBP in plasma of rainbow subjected to a handling disturbance (A) and nonstressed (B) animals. Stressed animals were subjected to a 5-min handling disturbance (netting and chasing), whereas unstressed animals were not handled. Stressed animals were sampled at 0 (before stressor/handling), 1, 4, and 24 h after handling disturbance. Values in parenthesis (x-axis labels) represent the clock time at which the sampling time points occurred. To control for temporal variation, unstressed animals were sampled at 09:00, 10:00, 13:00, and 08:00 (the following day; clock time) and these times corresponded with sampling times in the handled (stressed) animals. Units are arbitrary densitometry units (ADU) ± SEM. There was a significant effect of sampling time on plasma levels of the 50-kDa IGFBP in stressed ($P = 0.036$; 1-way ANOVA) and unstressed ($P < 0.001$; 1-way ANOVA) control animals. Time points (groups) with different letters are statistically different ($P < 0.05$; FPLSD).

recently, Wilkinson and colleagues [9] observed no effect of a 30-min confinement stress on plasma GH levels in rainbow trout, which is similar to our finding, but the stressors and duration are different between our study and the aforementioned studies. Given such variable results, generalizations of a stressor effect on plasma GH are difficult, but such differences may be attributed to the varying stressors (chronic versus acute) employed, sampling time course, methodologies, and species and rearing history of the animals used. In the present study, the handling stressor also elicited a transient elevation in plasma cortisol and glucose levels that recovered over a 24-h period in trout [47]. This plasma profile for cortisol and glucose in response to an acute stressor regimen (where the short-term stressor is removed and the animal is allowed to recover) is well established in teleosts and is a widely recognized measurement for stress [51,52].

In this study, we observed a transient reduction in plasma IGF-I levels at 1 and 4 h poststress with levels recovering by 24 h in rainbow trout. A similar plasma IGF-I response was also seen after a 15-min confinement stressor followed by recovery (where cortisol was transiently elevated and recovered within a 24-h period) in sunshine bass [45], but not in rainbow trout that were confined for 30–60 min [9]. Although a role for cortisol in the regulation of IGF-I levels, post-stressor exposure, cannot be ruled out, no study has yet established a direct link between cortisol stimulation and IGF regulation during stress in fish. Indeed, long-term cortisol treatment has been shown to affect plasma IGF-I and IGFBP levels in tilapia [53], hybrid bass [45], and channel catfish [54], suggesting a role for this steroid in the stressor-mediated regulation of the distal components (IGF-I and IGFBPs) of the somatotropic axis in fish. Further studies involving glucocorticoid receptor antagonists may be needed to indicate a stronger link between stress-induced cortisol stimulation and plasma IGF-I (and IGFBP) levels.

The role of IGFBPs in the regulation of plasma IGF-I levels and tissue responsiveness to this anabolic hormone during stress is still unclear. Furthermore, the physiological basis that underlies the decrease in plasma levels of the high-molecular-mass IGFBPs in unstressed trout is unclear. Despite this paucity of information and the need for further work in this area, some plausible explanations can be offered to partially explain the time-dependent decrease in the high-molecular-mass IGFBPs in unstressed trout in this study. Fasting and nutritional manipulations have demonstrated the differing metabolic controls over the high- and low-molecular-weight IGFBPs in fish [14,29,55]. Using a fasting and refeeding paradigm in coho salmon, Shimizu and co-workers [11] recently showed that plasma IGFBPs maintain their ability to respond rapidly to a feeding event (in fed and fasted animals), which suggests that IGFBPs may be sensitive indicators of nutritional status in finfish. In the animals from this study, food was withheld for a 48-h duration, which could explain the progressive decrease in plasma levels of the 42- and 50-kDa IGFBP in unstressed control rainbow trout from this study. By contrast, plasma levels of the 21- and 32-kDa IGFBPs in unstressed rainbow trout from this study were relatively stable over the 24-h sampling interval. Our finding is in agreement with that of Shimizu and co-workers [11], who generally found that levels of the 41-kDa IGFBP were lower in fasted animals, but dissimilar in that levels of IGFBP-I were elevated in 1-d fasted coho salmon, which was not seen in the rainbow trout from this study. Overall, rapid modulation of plasma IGFBPs levels by the nutritional state in coho salmon suggests a key role for these proteins in the metabolic adjustments to acute food limitation in salmonids.

Interestingly, acute stressor exposure offset the (food-deprivation or temporal) decrease in the high-molecular-mass IGFBPs seen in unstressed trout. The transient increase in the 50-kDa IGFBP at 1 h after stress correlates with the elevated plasma cortisol profile seen in these fish. Although the mechanism involved in this stress response is unknown, we hypothesize a role for cortisol in modulating the 42- and 50-kDa IGFBPs in trout, which is also supported by the findings that stress or cortisol did elevate plasma low-molecular-mass IGFBPs in hybrid bass [45], channel catfish [54], and tilapia [53], although such a link has not been established for cortisol or stressor effect on the high-molecular-mass IGFBPs in salmonids.

Because animals from the stressed and unstressed groups were limited to feed for identical periods of time, another possible explanation for the decrease in plasma levels of the high-molecular-mass IGFBPs (42 and 50 kDa) in unstressed animals is the presence of a temporal pattern. As mentioned above, these patterns (temporal or nutritional) appeared to be masked by a cortisol-mediated acute stressor (<5 min). This masking effect is manifested as elevated levels (particularly IGFBP-42K) of the higher-molecular-weight IGFBPs and a transient reduction in plasma IGF-I, both of which are anabolic components of the somatotropic axis in rainbow trout. The picture that is emerging is that the plasma IGF-I levels are rapidly modulated by

acute stressors and that this response correlates negatively with elevated cortisol levels. For instance, an acute confinement stressor reduced plasma IGF-I levels rapidly in hybrid bass and this response followed a plasma cortisol profile that was transiently elevated and recovered to unstressed levels over a 24-h period [45,56]. On the other hand, a chronic stressor that elevated cortisol levels for longer periods continued to result in reduced IGF-I levels for over 48 h in gilthead sea bream [44]. Taken together, acute stressor–mediated transient elevation of cortisol levels likely play a role in the regulation of plasma IGF-I and high-molecular-mass IGFBP levels (42 and 50 kDa) in rainbow trout. The opposing plasma response leads us to propose that acute stress regulation of plasma IGF-I may occur independently of high-molecular-mass IGFBPs, although we cannot rule out a role for these binding proteins in the target tissue response to this peptide during acute stress adaptation in rainbow trout.

## Acknowledgments

The authors gratefully acknowledge the technical support of Carol Coyle and Lisa Radler (USDA/ARS) and critical comments by Dr. Gregory Weber and anonymous reviewers. The study was funded by grants from the National Research Initiative Competitive Grants Program/USDA Award No. 2002-35206-11629 and 2004-05124 to B.S.S. and the USDA/ARS CRIS Project No. 3655-31320-001-00D and the Natural Sciences and Engineering Research Council of Canada Discovery grant to M.M.V. The views contained in this document are those of the authors and should not be interpreted as necessarily representing the official policies, either expressed or implied, of the U.S. Government. Mention of trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the USDA and does not imply its approval to the exclusion of other products that may be suitable. The manuscript was submitted for publication with the understanding that the U.S. Government is authorized to reproduce and distribute reprints for governmental purposes.

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